

Comparative Metabolism of Chloroacetamide Herbicides and Selected Metabolites in Human and Rat Liver Microsomes.

Scott Coleman,¹ Russell Linderman,² Ernest Hodgson,¹ and Randy L. Rose¹

¹Department of Toxicology; ²Department of Chemistry, North Carolina State University, Raleigh, North Carolina, USA

Acetochlor [2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methyl-phenyl)-acetamide], alachlor [*N*-(methoxymethyl)-2-chloro-*N*-(2,6-diethyl-phenyl)acetamide], butachlor [*N*-(butoxymethyl)-2-chloro-*N*-(2,6-diethyl-phenyl)acetamide], and metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl) acetamide] are pre-emergent herbicides used in the production of agricultural crops. These herbicides are carcinogenic in rats: acetochlor and alachlor cause tumors in the nasal turbinates, butachlor causes stomach tumors, and metolachlor causes liver tumors. It has been suggested that the carcinogenicity of these compounds involves a complex metabolic activation pathway leading to a DNA-reactive dialkylbenzoquinone imine. Important intermediates in this pathway are 2-chloro-*N*-(2,6-diethylphenyl)acetamide (CDEPA) produced from alachlor and butachlor and 2-chloro-*N*-(2-methyl-6-ethylphenyl)acetamide (CMEPA) produced from acetochlor and metolachlor. Subsequent metabolism of CDEPA and CMEPA produces 2,6-diethylaniline (DEA) and 2-methyl-6-ethylaniline (MEA), which are bioactivated through para-hydroxylation and subsequent oxidation to the proposed carcinogenic product dialkylbenzoquinone imine. The current study extends our earlier studies with alachlor and demonstrates that rat liver microsomes metabolize acetochlor and metolachlor to CMEPA (0.065 nmol/min/mg and 0.0133 nmol/min/mg, respectively), whereas human liver microsomes can metabolize only acetochlor to CMEPA (0.023 nmol/min/mg). Butachlor is metabolized to CDEPA to a much greater extent by rat liver microsomes (0.045 nmol/min/mg) than by human liver microsomes (< 0.001 nmol/min/mg). We have determined that both rat and human livers metabolize both CMEPA to MEA (0.308 nmol/min/mg and 0.541 nmol/min/mg, respectively) and CDEPA to DEA (0.350 nmol/min/mg and 0.841 nmol/min/mg, respectively). We have shown that both rat and human liver microsomes metabolize MEA (0.035 nmol/min/mg and 0.069 nmol/min/mg, respectively) and DEA (0.041 nmol/min/mg and 0.040 nmol/min/mg, respectively). We have also shown that the cytochrome P450 isoforms responsible for human metabolism of acetochlor, butachlor, and metolachlor are CYP3A4 and CYP2B6. **Key words:** chloroacetamide herbicides, human cytochrome P450, *in vitro* metabolism. *Environ Health Perspect* 108:1151–1157 (2000). [Online 7 November 2000]

<http://ehpnet1.niehs.nih.gov/docs/2000/108p1151-1157coleman/abstract.html>

Four chloroacetamide herbicides, acetochlor [2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide], alachlor [2-chloro-*N*-(methoxymethyl)-*N*-(2,6-diethylphenyl)acetamide], butachlor [*N*-(butoxymethyl)-2-chloro-*N*-(2,6-diethylphenyl)-acetamide], and metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide] (Figure 1), are widely used in the production of several economically important crops. Alachlor has an estimated annual use of 25 million pounds per year in the production of corn, soybeans, peanuts, and grain sorghum, and acetochlor has an annual estimated use of 35 million pounds per year, mostly in the production of corn. Metolachlor has an annual estimated use of 65 million pounds per year in the production of corn and soybeans and also for nonagricultural use in rights-of-way (1). Butachlor is not used in the United States but it is used in rice-producing countries.

The mutagenicity of acetochlor, alachlor, butachlor, and metolachlor has been reviewed thoroughly by Dearfield et al. (2). Both acetochlor (3–5) and alachlor (6–9)

have been found to cause increases in nasal tumors in rats, whereas metolachlor produces an increase in the incidence of liver tumors (10–13). Butachlor was found to be mutagenic in primary rat tracheal epithelial cells and Chinese hamster ovarian cells (14,15). The hypothesized mechanism of carcinogenicity is bioactivation of these herbicides through several steps, ultimately leading to the formation of a DNA-reactive metabolite (Figure 2), 2-methyl-6-ethylbenzoquinone imine (MEBQI) for acetochlor and metolachlor and 2,6-diethylbenzoquinone imine (DEBQI) for alachlor and butachlor. Acetochlor and metolachlor have been shown to form MEBQI, and alachlor and butachlor have been shown to form DEBQI in rats that were treated with these herbicides (16).

The Food Quality Protection Act of 1996 (17) includes a mandate for the U.S. Environmental Protection Agency (U.S. EPA) to consider cumulative risks of pesticides that have a common mechanism of action. The carcinogenic potential of the chloroacetamide herbicides, combined with the presence of a common metabolic

activation pathway leading to the formation of a putative ultimate carcinogenic metabolite, suggests that these herbicides have a common mechanism of carcinogenicity. If humans are able to metabolize each of these herbicides to the DNA-reactive metabolite, then the risk of these herbicides should be combined into the same risk cup. However, if humans are unable to bioactivate each of these herbicides, then the risk of each pesticide should be considered on an individual basis.

There are two main purposes of the present study: *a*) to determine the rates of metabolism of acetochlor and metolachlor to 2-chloro-*N*-(2-methyl-6-ethylphenyl)acetamide (CMEPA), butachlor to 2-chloro-*N*-(2,6-diethylphenyl)acetamide (CDEPA), CMEPA to 2-methyl-6-ethylaniline (MEA), CDEPA to 2,6-diethylaniline (DEA), MEA to 2-methyl-6-ethylaminophenol, and DEA to 2,6-diethylaminophenol using both rat and human liver microsomes; and *b*) to determine which human cytochrome P450 isoforms are responsible for the metabolism of these compounds. With this information, a more accurate risk assessment for these herbicides may be possible.

Materials and Methods

Chemicals. We purchased acetochlor, butachlor, and metolachlor from Chem Services (West Chester, PA); β -nicotinamide adenine dinucleotide phosphate (NADP), D-glucose-6-phosphate, and glucose-6-phosphate dehydrogenase from Sigma (St. Louis, MO); and diethylaniline, methylethylaniline, and chloroacetyl chloride from Aldrich (Milwaukee, WI). All other chemicals were the highest grade available and purchased from regional suppliers.

Metabolite synthesis. CMEPA was synthesized in 67% yield by acylation of methylethylaniline with chloroacetyl chloride in refluxing ethyl acetate using the method of Nesnow et al. (18). The structure of CMEPA was confirmed using nuclear

Address correspondence to R.L. Rose, Department of Toxicology, P.O. Box 7633, North Carolina State University, Raleigh, NC 27695 USA. Telephone: (919) 515-4378. Fax: (919) 515-7169. E-mail: randy_rose@ncsu.edu

This work was supported by the North Carolina Department of Agriculture Environmental Trust Fund and the National Institute of Environmental Health Sciences grant T32 ES07046.

Received 5 May 2000; accepted 1 August 2000.

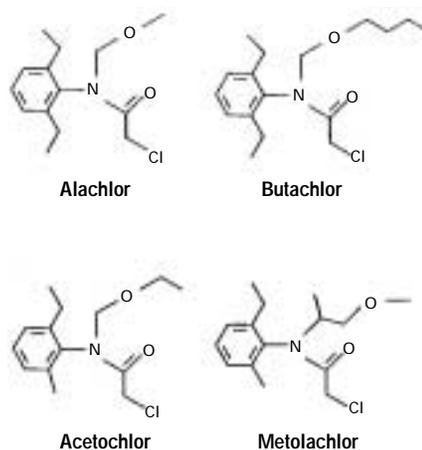


Figure 1. Chemical structure of acetochlor, alachlor, butachlor, and metolachlor.

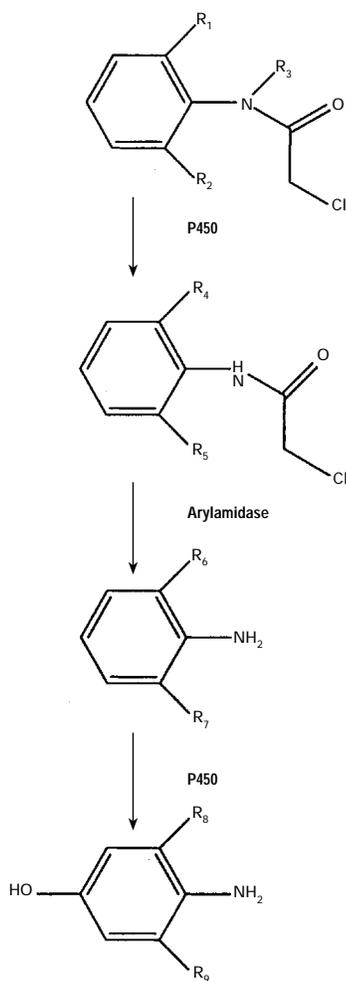


Figure 2. Metabolic pathway for the bioactivation of chloroacetamide herbicides. Acetochlor: R₁, CH₂CH₃; R₂, CH₃; R₃, CH₂OCH₂CH₃. Alachlor: R₁, CH₂CH₃; R₂, CH₂CH₃; R₃, CH₂OCH₃. Butachlor: R₁, CH₂CH₃; R₂, CH₂CH₃; R₃, CH₂O(CH₂)₃CH₃. Metolachlor: R₁, CH₂CH₃; R₂, CH₃; R₃, CH₃CHCH₂OCH₃. CDEPA: R₄, CH₂CH₃; R₅, CH₂CH₃. CMEPA: R₄, CH₂CH₃; R₅, CH₃. DEA: R₆, CH₂CH₃; R₇, CH₂CH₃. MEA: R₆, CH₂CH₃; R₇, CH₃. DEAOH: R₈, CH₂CH₃; R₉, CH₂CH₃. MEOH: R₈, CH₂CH₃; R₉, CH₃.

magnetic resonance (NMR) (Figure 3). CDEPA was synthesized as previously reported (19).

Microsome preparation. Microsomes were prepared from male Long-Evans rats (Charles River Laboratories, Raleigh, NC). Briefly, the animals were sacrificed and the livers were removed, weighed, and placed in ice-cold 50 mM potassium phosphate (pH 7.5) containing 0.1 mM EDTA and 1.15% potassium chloride. The livers were minced with scissors, homogenized with a Polytron homogenizer (Kinematica, Kriens-Lucern, Switzerland), and centrifuged at 10,000g for 15 min. The supernatant was filtered through glass wool and centrifuged at 100,000g for 1 hr. The microsomal pellet was resuspended in 50 mM potassium phosphate (pH 7.5) containing 0.1 mM EDTA and 0.25 M sucrose, and aliquots were stored at -80°C until use. We determined protein concentration using a BCA kit (Pierce Chemical Co., Rockford, IL) prior to use. Male human liver microsomes (20 mg/mL protein) were purchased from Xenotech, LLC (Kansas City, KS).

Human CYP isoforms. We purchased human CYP1A1, 1A2, 2B6, 2D6, 2E1, and 3A4 from Gentest Corporation (Woburn, MA). These isoforms are derived from microsomes of lymphoblastoma cell lines transfected with human cDNA for each isoform individually. Isoforms tested in our laboratory using assays suggested by Gentest had activities similar to those reported by Gentest.

Acetochlor, butachlor, metolachlor, MEA, and DEA metabolism. For the metabolism of each substrate by liver microsomes, CYP1A2, 2B6, 2D6, 2E1, or 3A4 isoforms, the substrate (50 μM) was incubated with the corresponding system (1 mg protein for the isoforms, human liver microsomes, and rat liver microsomes) in the presence of an NADPH regenerating system (0.25 mM NADP⁺, 2.5 mM glucose-6-phosphate, and 2.0 U glucose-6-phosphate dehydrogenase) in 0.1 M phosphate buffer, 0.005 M MgCl₂ at pH 7.4 for up to 1 hr. For substrate metabolism by CYP2A6, we incubated the substrate (50 μM) with the isoform (1 mg protein) in the presence of an NADPH regenerating system (0.25 mM NADP⁺, 2.5 mM glucose-6-phosphate, and 2.0 U glucose-6-phosphate dehydrogenase) in 0.1 M Tris buffer, 0.005 M MgCl₂ at pH 7.5 for up to 1 hr. Aliquots were removed at time points up to 20 min and added to an equal volume of ice-cold methanol. The solution was then vortexed and centrifuged. The supernatant was injected directly onto an HPLC consisting of a reversed-phase column (Prodigy C18, 250 × 3.9 mm; Phenomenex, Torrance, CA) with a mobile phase of 55:45 (v/v) water:acetonitrile with UV detection at 238 nm. The limit of detection for CMEPA, CDEPA, MEA, and DEA was 0.0625 nmol. Rates of CMEPA or CDEPA formation were calculated against a standard curve for either CDEPA or CMEPA that we ran on the same day. Rates

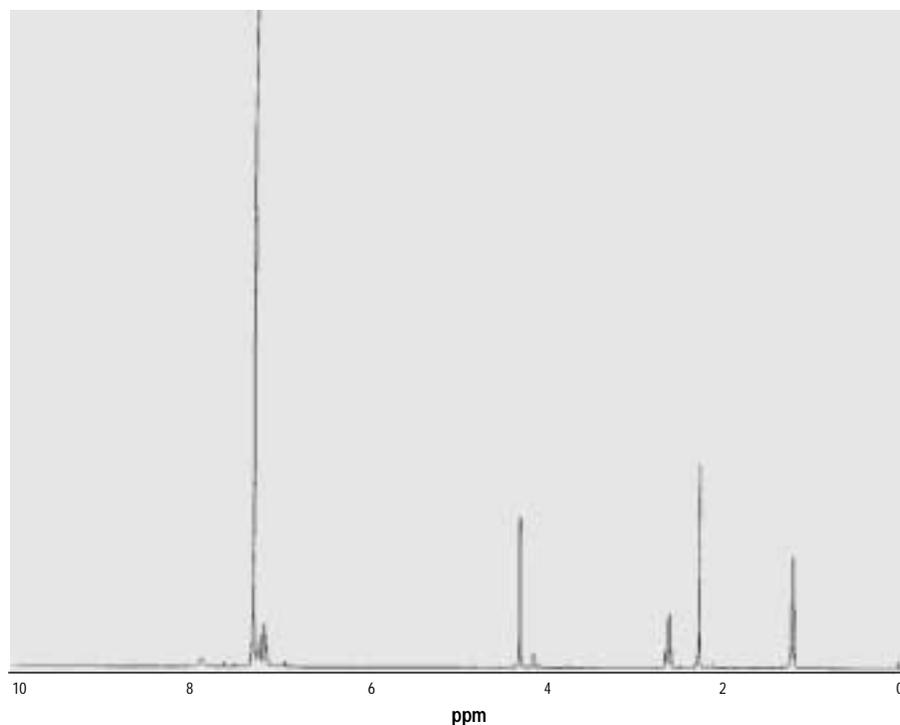


Figure 3. ¹H-NMR spectra of CMEPA.

of MEA or DEA oxidation were calculated as the loss of substrate against a standard curve for either MEA or DEA that we ran on the same day.

CMEPA and CDEPA Metabolism. For the metabolism of each of these compounds by rat and human liver microsomes, the substrate (50 μ M) was incubated with protein (2 mg for rat liver microsomes and 0.2 mg for human liver microsomes) in 0.1 M phosphate buffer, 0.005 M $MgCl_2$, pH 7.4, for up to 1 hr. Aliquots were removed at time points up to 1 hr and added to an equal volume of ice-cold methanol. The solution was then vortexed and centrifuged. The supernatant was injected directly onto an HPLC consisting of a reversed phase column (Prodigy C18, 250 \times 4.6 mm; Phenomenex) with a mobile phase of 55:45 (v/v) water:acetonitrile with UV detection at 238 nm. Rates of MEA or DEA formation were calculated against a standard curve for either MEA or DEA ran on the same day. The K_m and V_{max} values for rat and human liver microsomal arylamidase toward CDEPA was calculated using a Lineweaver-Burke double reciprocal plot.

Results

We synthesized CMEPA by following the method of Nesnow (18) for CDEPA synthesis. The structure of CMEPA was confirmed using 1H -NMR spectroscopy (Figure 3).

Figures 4 and 5 summarize the results of rat and human liver microsomal metabolism of acetochlor, alachlor, butachlor, metolachlor, CDEPA, CMEPA, DEA, and MEA. As previously observed for alachlor (19), the most striking differences in the metabolism were observed between species in the initial step leading to the production of CDEPA (butachlor) or CMEPA (acetochlor or metolachlor). For acetochlor, CMEPA production by rat liver microsomes was similar to that of human liver microsomes (0.065 nmol/min/mg and 0.023 nmol/min/mg, respectively), whereas a more pronounced difference was observed for butachlor (0.045 nmol/min/mg and < 0.0015 nmol/min/mg, respectively). In contrast, rat liver microsomes metabolized metolachlor to CMEPA at a rate of 0.013 nmol/min/mg, whereas human liver microsomes were unable to metabolize metolachlor to CMEPA (Figures 6 and 7). When acetochlor, alachlor, butachlor, and metolachlor were incubated with either rat or human liver microsomes for up to 20 min, we were not able to detect any aniline, DEA, or MEA formation. This suggests that any CDEPA or CMEPA produced in this time was either not being metabolized to the corresponding aniline by the aryl amidase or metabolized so slowly as to preclude detection.

Other differences in the metabolism relating to the subsequent steps in the pathway

leading to the formation of the DABQI were minimal between species. Although both rats and humans metabolized CDEPA to DEA (Figures 8 and 9, respectively), the human liver microsomal arylamidase was more active toward either CDEPA or CMEPA than the rat liver microsomal arylamidase. These species differences are reflected by the different K_m and V_{max} values obtained from rat liver microsomal arylamidase (230 μ M and 1.2 nmol/min/mg, respectively) and human liver microsomal arylamidase values (470 mM and 9.1 nmol/min/mg, respectively). We observed no major differences between species in the metabolism of DEA or MEA to their respective hydroxylated products (Figures 4 and 5).

We also determined that acetochlor, butachlor, and metolachlor are metabolized

by CYP2B6 and 3A4. In previous work (19), we demonstrated that alachlor was metabolized by CYP3A4, but not by several other isoforms including CYP2B6. Reanalysis of CYP2B6 with respect to alachlor revealed that alachlor is a substrate for this isoform.

CYP2B6 metabolizes the substrates to only one product, whereas CYP3A4 is able to metabolize the substrates to multiple products, as shown for metolachlor in Figures 10 and 11. CYP2B6 metabolized both alachlor and butachlor to CDEPA, acetochlor to CMEPA, and metolachlor to an unknown metabolite. Because CYP2B6 N-dealkoxylated acetochlor, alachlor, and butachlor, we thought that the unknown metabolite was a derivative of the possible N-dealkoxylated product. To determine the

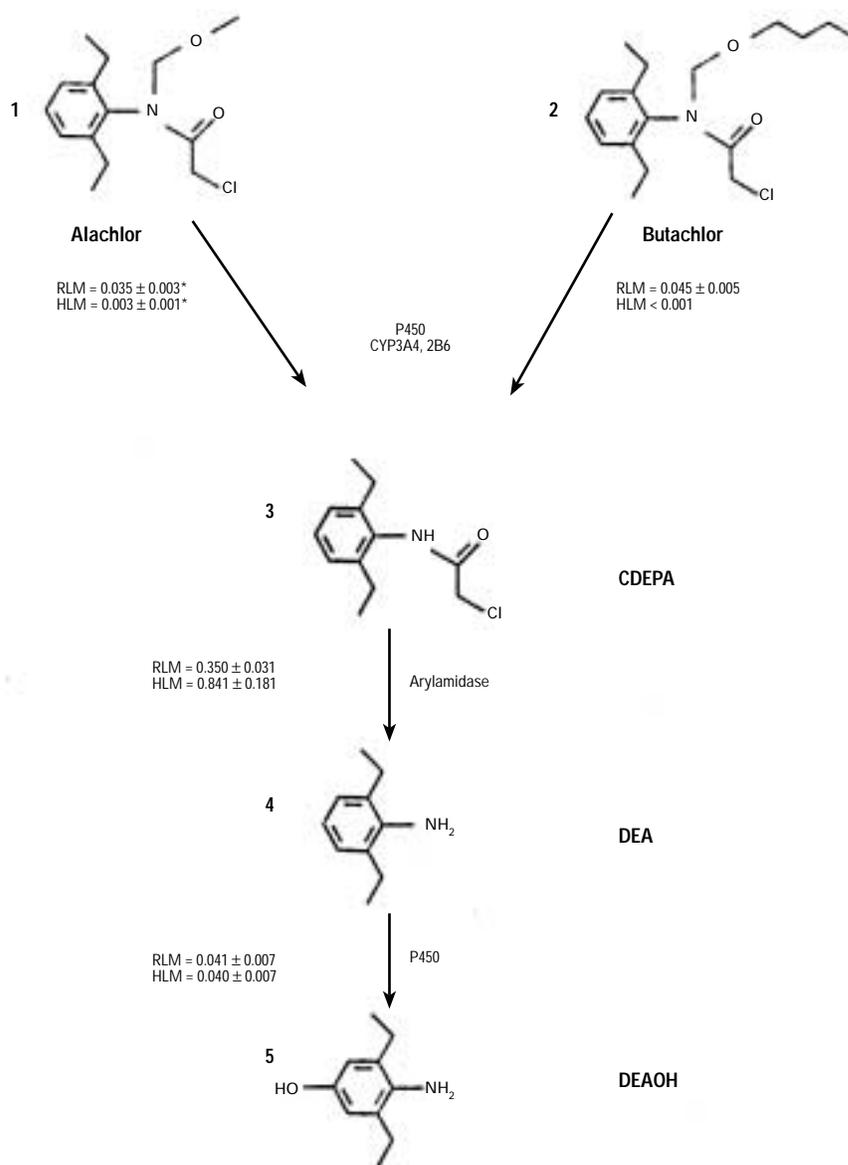


Figure 4. Comparison of the metabolism of alachlor, butachlor, CDEPA, and DEA between rat and human liver microsomes. Abbreviations: RLM, rat liver microsomes; HLM, human liver microsomes. Rates are expressed as nmol/min/mg \pm SEM.

*Data from Coleman et al. (19).

structure of this metabolite we used liquid chromatography/mass spectrometry with Atmospheric Pressure Chemical Ionization (APCI) in the positive mode. We were able to determine that the metabolite had a base peak of 270 m/z while metolachlor had a base peak of 284 m/z , suggesting *O*-demethylation of metolachlor leading to the formation of 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-hydroxy-1-methylethyl)-acetamide.

Discussion

The U.S. EPA has classified alachlor and acetochlor as probable human carcinogens and metolachlor as a possible human carcinogen; there has been no classification of butachlor at present. The classification of alachlor and acetochlor as probable human carcinogens and metolachlor as a possible human carcinogen are based on the findings of increased

incidence of tumors in rats chronically administered these herbicides. One proposed mechanism of carcinogenicity for acetochlor, alachlor, and metolachlor involves their bioactivation to a DNA-reactive metabolite. Although metabolism of acetochlor, alachlor, butachlor, and metolachlor has been extensively studied in rats, there has been minimal work examining the metabolism of these herbicides in humans. Earlier work demonstrated that metabolites of alachlor formed DNA adducts (20) as well as DNA single strand breaks (21). Recent studies have indicated that rats are able to metabolize acetochlor, alachlor, butachlor, and metolachlor to their putative DNA-reactive benzoquinone imine products (16).

The formation of DNA-reactive metabolites from acetochlor, alachlor, butachlor, and metolachlor is dependent on the production

of either DEA or MEA. There are two possible ways for these anilines to be formed: through the formation of CDEPA/CMEPA by P450 followed by an arylamidase reaction, or through conjugation of the herbicide with glutathione by displacement of the chlorine atom and subsequent amide hydrolysis. Li et al. (22) suggested that the formation of DEA from alachlor in rats and monkeys is dependent upon the conjugation of alachlor to glutathione, consequent enterohepatic circulation of this reduced glutathione (GSH) conjugate, conversion of the GSH conjugate to the secondary methylsulfide, and amide hydrolysis of the secondary methylsulfide to DEA (22). It was suggested that because this occurs in monkeys at a slow rate, this conversion would also occur at a slow rate in humans. Heydens et al. (23) showed that when an *in vitro* model for rats and humans is used, alachlor is conjugated to GSH at a rate in humans approximately four times less than in rats (23). With this information it appears that alachlor may not be as good a substrate with human GSH transferases as compared to rat GSH transferases. Because conjugation of alachlor with GSH occurs slowly in humans, alachlor may be available as a substrate for human P450s leading to the formation of CDEPA.

In the current study, incubations of the parent herbicides with liver microsomes resulted in major differences between rats and humans in the formation of CDEPA or CMEPA. Our data also indicate that the arylamidase activity in microsomal tissues of both organisms is not rate-limiting with respect to DEA or MEA formation. It could be argued that the presence of the arylamidase in microsomes effectively removes CDEPA or CMEPA as quickly as it is formed, resulting in apparently low steady-state levels of CDEPA or CMEPA product formation. However, formation of DEA or MEA was not observed during microsomal incubations up to 20 min, and in very low amounts in incubations exceeding 1 hr. This may be due to the fact that the concentrations of CDEPA or CMEPA were well below saturation; thus further metabolism under the conditions of the assay would be expected to be slow. This is supported by the high K_m values reported for the rat and human arylamidases (231 and 473 μM , respectively).

For acetochlor, the rate of metabolism to CMEPA by human liver microsomes is approximately one-third the rate observed in rat liver microsomes. Although rat liver microsomes metabolized metolachlor to CMEPA at a rate approximately one-fifth the rate observed for acetochlor, human liver microsomes were unable to form CMEPA from metolachlor. Likewise in human liver microsomes, the rate of metabolism of

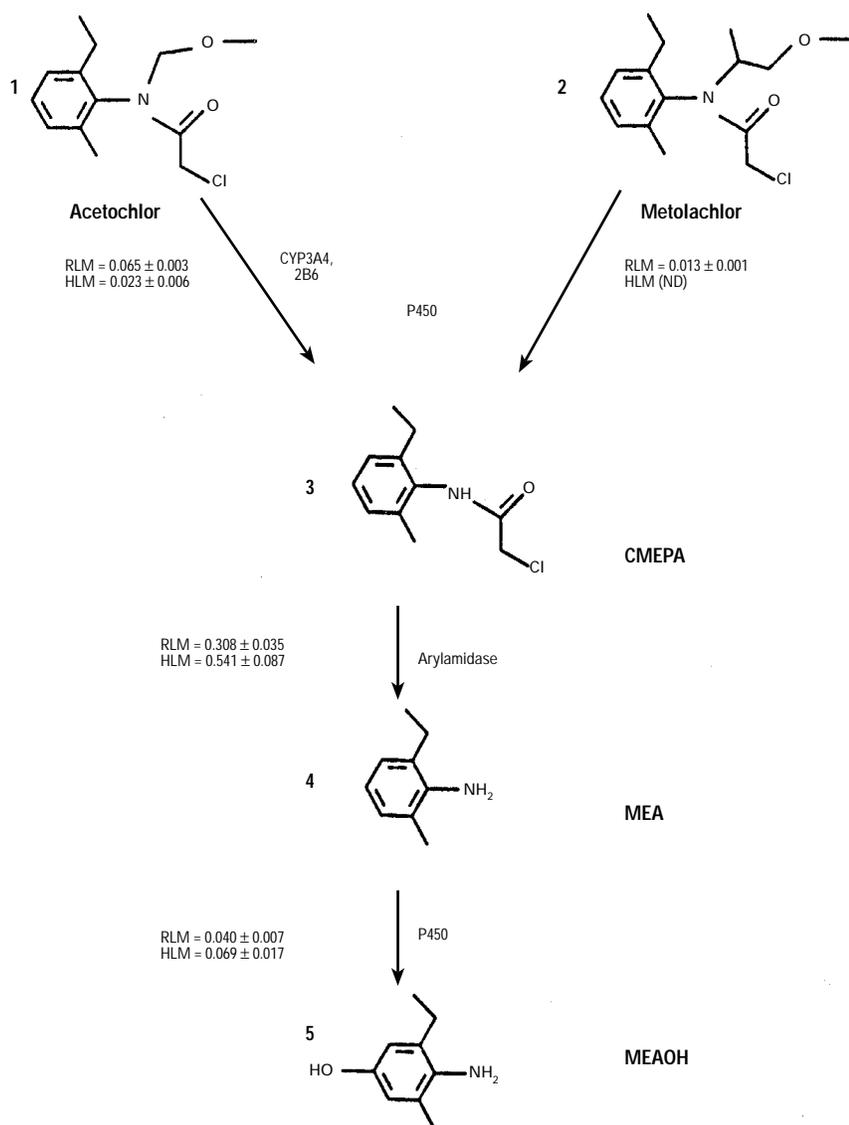


Figure 5. Comparison of the metabolism of acetochlor, metolachlor, CMEPA, and MEA between rat and human liver microsomes. Abbreviations: HLM, human liver microsomes; ND, none detected; RLM, rat liver microsomes. Rates are expressed as nmol/min/mg \pm SEM.

butachlor to CDEPA was significantly less than the rate observed with rat liver microsomes. Our recent studies with alachlor also demonstrated low rates of metabolism to CDEPA by human liver microsomes as compared to the rate for rat liver microsomes (19). The lower rates of CDEPA formation from alachlor and butachlor by human liver microsomes suggest that there would be less DEA formed. Metolachlor is not metabolized to CMEPA by human liver microsomes, which suggests that no MEA would be formed even if metolachlor were conjugated to GSH. Feng et al. (24) showed that for compounds to be a substrate of the microsomal arylamidase, there cannot be any substitution on the nitrogen.

The ability of these herbicides to be substrates for both CYP2B6 and CYP3A4 is supported by the molecular modeling of CYP 3A4 by Szklarz and Halpert (25). They showed that some, but not all, of the residues that are important in the interaction of substrates with CYP3A are also located within rat CYP2B. This may explain why CYP2B6 produces only one metabolite, but CYP3A4 produces multiple metabolites. In

our previous work using a less sensitive method, we reported that alachlor was a substrate for CYP3A4 only (19) but here we show that alachlor is metabolized by CYP2B6.

It also appears that there are some structural requirements for the metabolism of these herbicides. Because metolachlor is O-demethylated and not N-dealkoxylated, this suggests that the N-alkoxy group cannot be bulky. Also, the slow rate of metabolism of butachlor to CDEPA suggests that the length of the alkoxy group needs to be less than six atoms. The greater metabolism of acetochlor to CMEPA as compared to the metabolism of alachlor to CDEPA suggests that the methylethyl phenyl group is more readily metabolized than the diethyl phenol group in these pesticides.

The liver microsomal arylamidase responsible for the metabolism of both CDEPA and CMEPA is also a carboxylesterase. There are several isoforms of carboxylesterases that have been classified based on substrate specificity and their isoelectric point (26). We have shown that there are differences in the ability of the rat and human liver microsomal

arylamidase to cleave CDEPA and CMEPA. The human liver microsomal arylamidase appears to metabolize CDEPA at a rate twice that of the rat liver microsomal arylamidase, whereas the activity of both the human and rat liver microsomal arylamidase toward CMEPA appear similar. The differences we have shown could be due to isoform specificity of the carboxylesterases between rats and humans. Previous work has suggested that human liver microsomes have very little activity toward CDEPA (23). We have clearly shown that both human and rat liver microsomes have activity toward CDEPA. To ensure that this activity was not due to nonenzymatic hydrolysis, we used negative controls in which the substrate was incubated in buffer without microsomes. No DEA was produced in these negative controls.

DEA is metabolized by rat liver microsomes to *p*-hydroxy-diethylaniline (27). Heydens et al. (23) showed that there are significant differences in the oxidation of DEA between rat and human liver microsomes, with rat liver microsomes having a 7–10-fold greater activity toward DEA than human liver microsomes. Our results contrasted

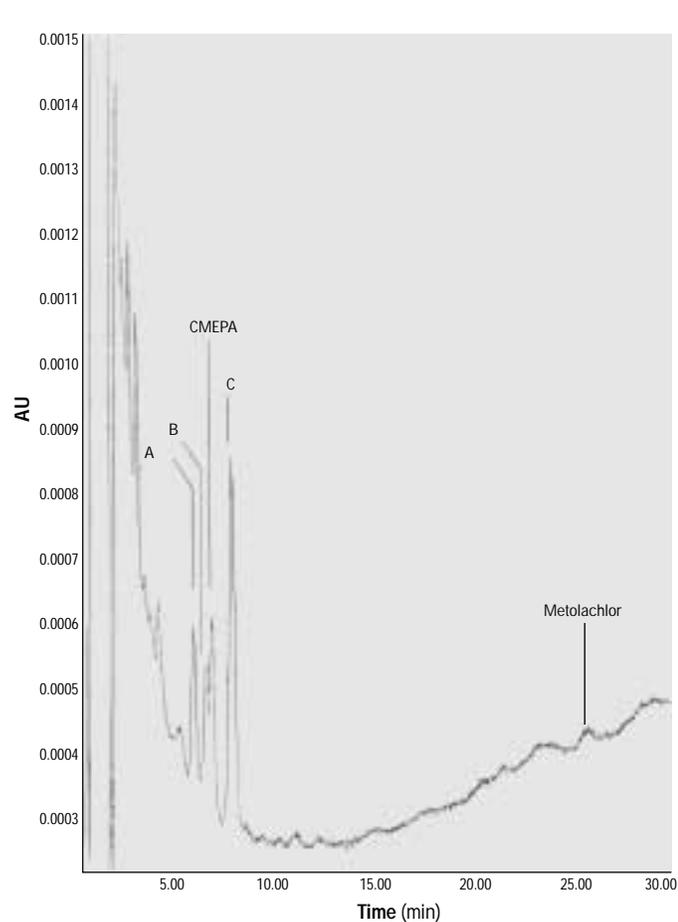


Figure 6. Metabolism of metolachlor (50 μ M) by rat liver microsomes (1 mg protein) at 37°C for 20 min. AU, absorbance units. Peaks A, B, and C are unknown metabolites.

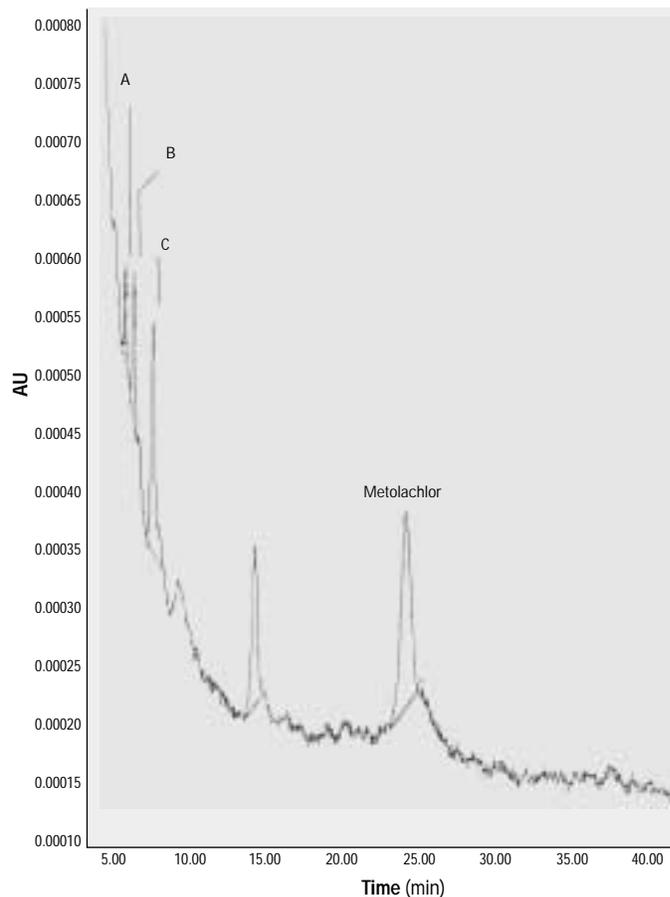


Figure 7. Metabolism of metolachlor (50 μ M) by human liver microsomes (1 mg protein) at 37°C for 20 min. AU, absorbance units. Peaks A and C are unknown metabolites; peak B is 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-hydroxy-1-methyl-ethyl)acetamide.

significantly in that we found very similar rates of metabolism of DEA by rat and human liver microsomes. However, we were able to see a difference in the rate of metabolism of MEA by rat and human liver

microsomes, with human liver microsomes having a rate of MEA oxidation almost twice that of rat liver microsomes.

The regulations of the Food Quality Protection Act of 1996 (17) suggest that

chemicals possessing the same mechanism of action should be combined into an aggregate group known as a risk cup. The combined human exposure of chemicals in the same risk cup must not exceed a limit yet to be

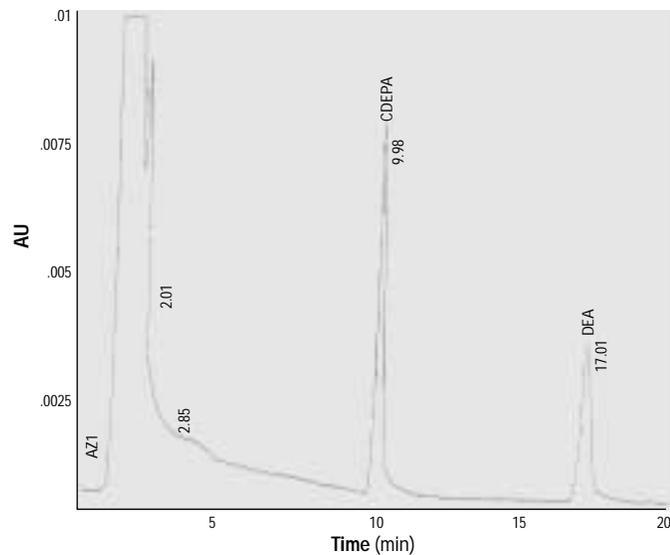


Figure 8. Metabolism of CDEPA (50 μ M) by rat liver microsomes (2 mg protein) at 37°C for 20 min. AU, absorbance units.

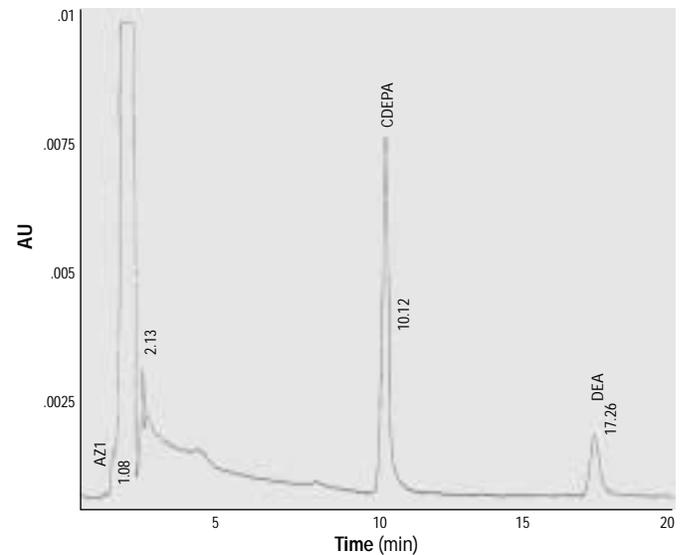


Figure 9. Metabolism of CDEPA (50 μ M) by human liver microsomes (0.2 mg protein) at 37°C for 20 min. AU, absorbance units.

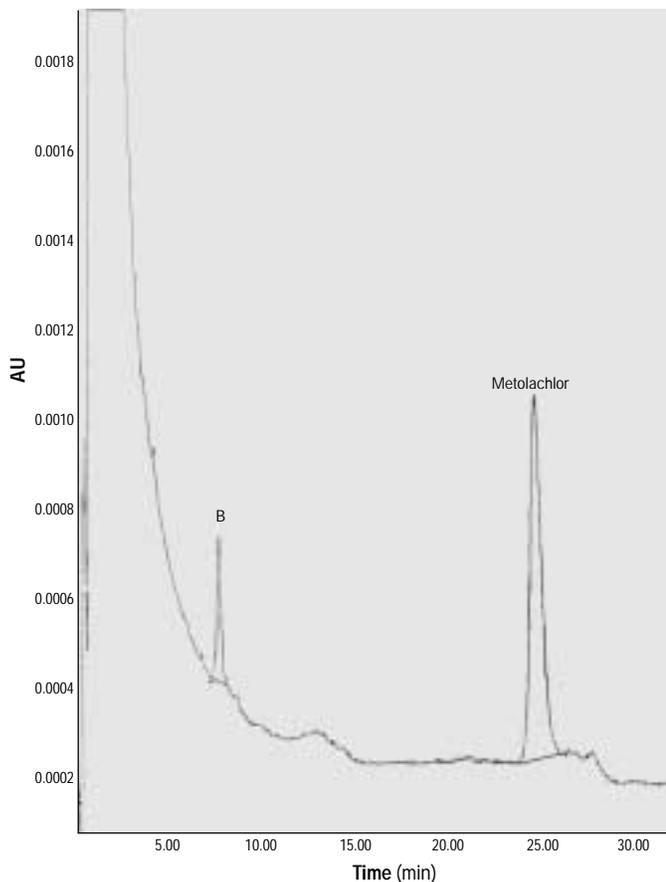


Figure 10. Metabolism of metolachlor (50 μ M) by human CYP2B6 (1 mg protein) at 37°C for 20 min. AU, absorbance units. Peak B is 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-hydroxy-1-methylethyl)acetamide.

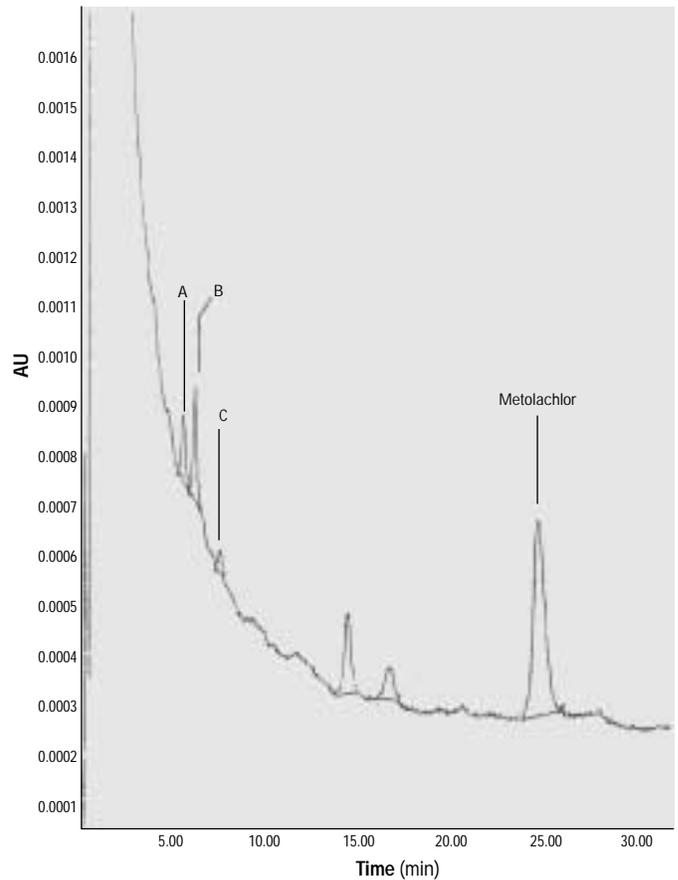


Figure 11. Metabolism of metolachlor (50 μ M) by human CYP3A4 (1 mg protein) at 37°C for 20 min. AU, absorbance units. Peaks A and C are unknown metabolites; peak B is 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-hydroxy-1-methylethyl)acetamide.

determined. It has been suggested that the chloroacetanilide herbicides should belong in the same risk cup based upon the proposed mechanism of carcinogenicity being bioactivation to the corresponding DABQI (28). We have shown, however, that there are significant differences in the ability of human liver microsomes to metabolize acetochlor, alachlor, butachlor, and metolachlor compared to rat liver microsomes. This implies that these herbicides may be divided into separate risk cups.

One aspect that the present study does not address is the extrahepatic metabolism of these compounds. Wetmore et al. (29) demonstrated that alachlor is mutagenic in the Ames assay, but only in the presence of Long-Evans olfactory mucosal S9. Further work examining the possible metabolism of the compounds used in the present study by human nasal tissue or other extrahepatic tissue would provide more insight for this issue.

In summary, our work using human liver microsomes suggests that metolachlor cannot be metabolized to CMEPA, a necessary precursor for the formation of the putative ultimate carcinogen MEBQI. Thus, based on our work with human liver microsomes, metolachlor may not be genotoxic to humans. Alachlor and butachlor are metabolized to CDEPA at substantially lower rates by human liver microsomes than by rat liver microsomes, which suggests that alachlor and butachlor may have minimal human genotoxicity. However, human liver microsomes metabolize acetochlor to CMEPA at a similar rate to that of rat liver microsomes, and subsequent metabolic rates of CMEPA and MEA with human liver microsomes exceed those of rat liver microsomes, suggesting that acetochlor has the greatest potential to be genotoxic to humans.

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